# Effects of Atrazine on Non-Target Organisms

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## Introduction

Atrazine is an herbicide used to control annual broad leaf and grass weeds in agriculture and landscape maintenance of residential and commercial settings (Warnemuende 2006). This herbicide affects electron transport in photosystem II disrupting the photosynthetic process of targeted weeds (Devine et al., 1993). 2-chloro-4-ethylamino-6-isopropyl-amino-1-s-triazine is the chemical name for atrazine (Solomon et al., 1996). Atrazine is prevalent in the environment (Warnemuende 2006). In addition to its intended effects on photosynthesis, atrazine has been shown in some scientific studies to negatively impact non-target organisms in the environment; including non-target plants, phytoplankton, and invertebrates. The following information in this literature review is intended to introduce readers to atrazine and the herbicide's possible impacts on non-target organisms.

Weeds are the most harmful pest that agriculture faces in terms of production. The need for tillage is reduced via chemical solutions to weed problems (Warnemuende et al., 2006). Hence, it is argued that in addition to agricultural production, soil conservation is enhanced by herbicide use (Warnemuende et al., 2006). Herbicide use is currently argued as a necessity to conserve resources such as soil by allowing for productive agriculture via chemical technology. Also, the use of herbicides allow for the output production level currently seen or achieved within the United States. However, atrazine is susceptible to runoff relatively easily. Surface waters have been found to contain atrazine in a majority of the United States where agriculture is prevalent (Capel et al., 2001 and Solomon et al., 1996).

Atrazine can contaminate environmental waters through various pathways and locations such as runoff/drainage of treated areas, precipitation, and pesticide water spills. Roughly 33 to 36 million kilograms of atrazine are sold to the United States each year (Tillitt et al., 2010). The economic impact of atrazine is a factor when taking into accounts the benefits and possible negative impacts that the chemical might create in the United States (Ackerman et al., 2007).

Another concern is the possibility of atrazine exposure to humans. Human exposure could potentially occur from drinking water, as well as pesticide handling. Many studies have evaluated source water, finished water, and distribution system water for atrazine contamination. Benotti et al., (2009) reported the largest contaminating chemical in source, finished, and distribution system water to be atrazine with concentrations, respectively, at 32 ng/L, 49 ng/L, and 49 ng/L, respectively. Thus, the environmentally relevant concentrations, or rather levels of atrazine that might occur in the environment due to unintended runoff from agricultural applications, can be estimated from the literature.

Studies have been conducted to evaluate the toxicological response of many different animals exposed to atrazine. There have been many breakthroughs and changes in the understanding of atrazine's possible impact to organisms in the environment. Atrazine has been evaluated to determine possible hazard to vertebrates and invertebrates that have the potential to be affected in an unintended exposure to the chemical.

**Chemical Properties**

The chemical properties of atrazine allow it to be susceptible to runoff from agricultural and urban application due to rainfall and poor irrigation management. According to Solomon et al (1996), due to the magnitude of atrazine use in the United States, atrazine concentrations of approximately 0.1 μg/L to 30 μg/L are environmentally relevant concentrations found in surface

water of United States. The vapor pressure of atrazine is 2.89 x 10^-7 mm at 25 °C, which by many standards is relatively low (Solomon et al., 1996). Henry's law constant for atrazine is 2.48 $^{-9}$ atm*m$^{3}$ mol$^{-1}$ which is considered low according to Solomon et al. (1996), which makes it more prone to inundate environmental waters.

Atrazine has a molecular weight of 215.70 g/mol and a melting point between 175-177 °C (Solomon et al., 1996). The water solubility of atrazine is 33 µg/mL at 22 °C (Solomon et al., 1996). Solomon et al. (1996) reported that the values of the vapor pressure and Henry's law constant made atrazine a relatively low risk for evaporating from surface water, which in turn would yield contaminated rainfall. Nevertheless, rain contaminated with trace concentrations of atrazine has been documented. For example, spring and summer rainfall has detectable amounts of atrazine in the Midwestern and Northeastern United States according to a USGS publication (Goolsby 1997).

The Log $K_{ow}$ is 2.68 at 25 °C (Solomon et al., 1996). Solomon et al. (1996) reported that atrazine was stable to hydrolysis for 30 days at pH 5-9 and 25 °C. The aqueous photolysis half-life (T ½) for atrazine with a natural light measured was 17.5 hours at pH 7, 12 days under a Mercury lamp, and 45 days under a Xenon lamp. Aerobic soil metabolism halflife was reported to be 146 days in a California loam. The anaerobic soil metabolism halflife was 77 days in a California sandy loam, and 159 days in a California loam (Solomon et al., 1996). The anaerobic aqueous metabolism halflife was reported to be 608 days in a Georgia sandy clay (Solomon et al., 1996).

The soil ($K_d$) and organic carbon partition, ($K_{oc}$), coefficients for atrazine and its major metabolites in a Maryland clay was reported as 2.46 and 87.0, respectively (Solomon et al., 1996). The major metabolites of atrazine include deethylatrazine, deisopropylatrazine,

diaminochlorotriazine, deethylhyroxyatrazine, and deisopropylhydroxyatrazine. These degradation products must also be considered as possible contaminants of environmental concern. Solomon et al. (1996) reported the $K_d$ as 2.45 and the $K_{oc}$ as 36.1 for deethylatrazine in a Maryland clay.

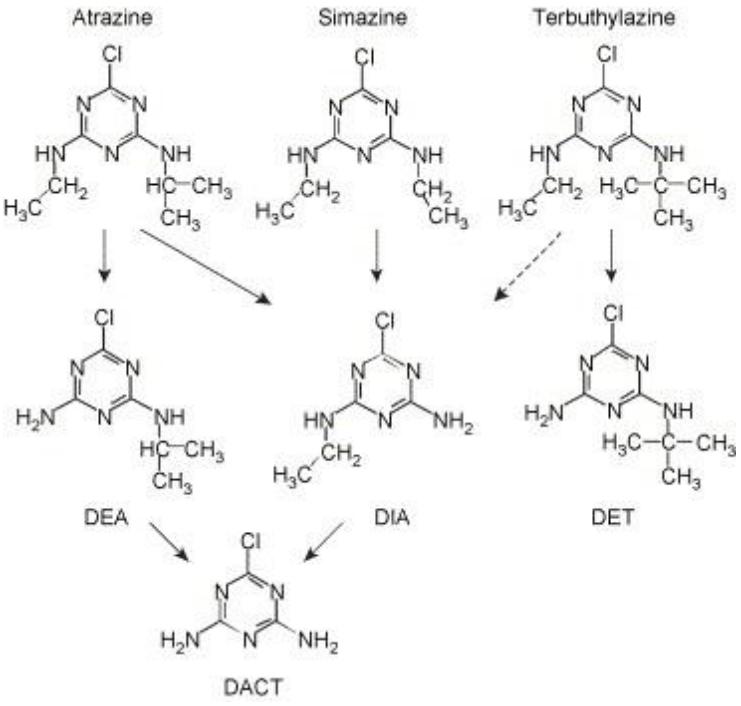


Figure 1. Figure depicting the metabolites of atrazine

(DuPreez et al., 2005)

| | ATZ | | DEA | | HA | | DIA | | DAC | |
|---|---|---|---|---|---|---|---|---|---|---|
| Soil type[b] | $K_d$ | $K_{oc}$ | $K_d$ | $K_{oc}$ | $K_d$ | $K_{oc}$ | $K_d$ | $K_{oc}$ | $K_d$ | $K_{oc}$ |
| MD clay | 2.46 | 87.0 | 1.02 | 36.1 | 389 | 13,797 | 2.73 | 97 | 1.56 | 55 |
| MD sand | 0.20 | 39.0 | 0.06 | 12.2 | 1.98 | 374 | 0.16 | 30 | 0.16 | 31 |
| MD sandy loam | 0.79 | 70.0 | 0.36 | 31.8 | 6.52 | 583 | 0.51 | 45 | 0.65 | 58 |
| CA loam | 0.73 | 155.0 | 0.21 | 44.9 | 12.1 | 2,573 | 0.27 | 58 | 0.36 | 76 |
| CA sandy loam | 0.19 | 25.3 | NA | NA | NA | NA | NA | NA | NA | NA |

[a] ATZ = atrazine, DEA = deethylatrazine, HA = hydroxyatrazine, DIA = deisopropylatrazine, DAC = diaminochlorotriazine.
[b] MD clay = 4.8% OM[1], 2.8% OC[2], pH 5.9; MD sand = 0.9% OM, 0.5% OC, pH 6.5; MD sandy loam = 1.9% OM, 1.1% OC, pH 7.5; CA loam = 0.8% OM, 0.5% OC, pH 6.7; CA sandy loam = 1.3% OM, 0.76% OC, pH 7.8. Source [11].

Table 1; reference (Solomon 1996)

Solomon et al. (1996) reported values for the $K_d$ and $K_{oc}$ for deethylatrazine (DEA), deisopropylatrazine (DIA), diaminochlorotriazine (DAC) , deethylhyroxyatrazine (DEH), and deisopropylhydroxyatrazine (DIP), and hydroxyatrazine (HA) within a Maryland clay, a Maryland sand, a Maryland sandy loam, a California loam, and a California sandy loam.

Deethlyatrazine originates solely from the herbicide atrazine, since it retains the isopropyl group (Du Preez et al., 2005). Du Preez et al., (2005) reported that concentrations of deethlyatrazine increased after crop production procedures such as planting and chemical applications. The concentrations of deethylatrazine correlated to the amount of atrazine used for the agricultural purposes that season (Du Preez et al., 2005). Deisopropyl atrazine, a major metabolite of atrazine, could be formed from other triazine herbicides such as simazine and terbuthylazine, since the ethyl group is retained (Du Preez et al., 2005). It is important to note that major metabolites of atrazine can also originate from other sources of triazine herbicides.

**Environmentally Relevant Concentrations**

Precipitation is one way in which atrazine can transfer from an area of intended application to another non target location. Vogel et al. (2008) reported that the samples detected atrazine in rain within agricultural areas.

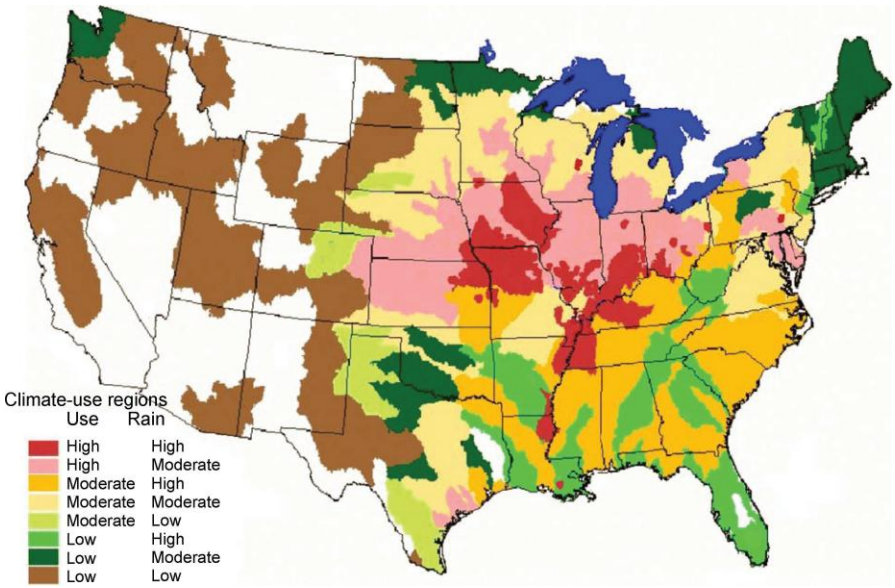


Figure 2.

Map from (Solomon et al., 2008)

Figure 2 is a map of rainfall and atrazine use analyzed for the United States (Solomon et al., 2008).

Environmentally relevant concentration may differ depending on the location in the watershed, land uses, environmental conditions, and whether water is flowing or static. The contaminated rainfall may affect pristine ecosystems that could be vulnerable to the low levels of atrazine. Non target organisms in the environment could be affected by atrazine exposure via contaminated precipitation (Vogel et al., 2008). The atrazine in rainfall was less than 2% of the total pesticides applied in the agricultural areas under consideration with respect to the Vogel et al., (2008). Amounts less than 2% of the total pesticides used in agricultural areas might be enough to possibly effect animal biological function, such as reproductive functions (Hayes et al., 2010). Miller et al. (2000) reported that atrazine was one of the most prevalent contaminants of rainfall in the Michigan lakes area with respect to the other contaminants under evaluation.

**Freshwater Phytoplankton**

Freshwater phytoplankton, commonly referred to as algae, have been evaluated to determine the effects of atrazine exposure. The LC50 is the median lethal concentration (Sutton et al., 1996). The lowest concentration causing an observed effect is the LOEC (Sutton et al., 1996). The NOEC is the highest concentration where there were no observable effects (Sutton et al., 1996). The EC50 is the median concentration affecting 50% of the measured process (Sutton et al., 1996). *Chlorella vulgaris* was found to have an EC50 of 25µg/L after 11 days (Solomon 1996 from Burrell 1985). Another study found the NOEC to equal 500 µg/L at an exposure duration of 7 days (Solomon 1996 from Veber 1981). The EC50 for *Chlorella vulgaris* was 42 µg/L to 53 µg/L after 24 hours of exposure. (Solomon 1996 from Larsen 1986).

*Chlorella pyrenoidosa* was found to have an EC50 of 125 after 24 hours (Solomon 1996 from Stratton 1990). Stratton et al., (1984) reported the degradates of atrazine to have an EC50 of 1800 mg/L for DEA and 3600 mg/L for DIA and potency ratios, (metabolite EC50)/(atrazine EC50), were calculated in the study (Stratton et al. 1984). It was reported that 55 µg/L of atrazine exposure reduced growth and $O_2$ production in *Chlorella pyrenoidosa* (Solomon 1996 from Gonzalez-Murua 1985). The EC50 for growth of *Chlorella pyrenidosa* was reported at 175µg/L following an exposure of 5 days (Solomon 1996 from Gramlich 1964). Photosynthesis inhibition was reported at an EC50 of 139 µg/L for *Chlorella pyrenidosa* (Solomon 1996 from Zweig 1963).

The following are examples of studies where the EC50 of *Ankistrodesmus braunii*, *Scenedesmus subspicatus*, and *Selenastrum capricornutum* were reported when exposed to atrazine for different durations. *Ankistrodesmus braunii* had a EC50 of 60 µg/L following 11 days exposure (Solomon et al., 1996 from Burrell et al., 1985). *Scenedesmus subspicatus* had an EC50 of 110 µg/L following 96 hours exposure (Solomon et al., 1996 from Geyer et al., 1985).

*Selenastrum capricornutum* had an EC50 at concentration level 214 μg/L following 7 days exposure (Solomon et al., 1996)

**Macrophytes**

Plants that are considered non-target have been studied in relation to atrazine effects. The possible impacts of atrazine exposure to non-target plants could affect valuable habitat and food for a variety of organisms. The (EC50), (LOEC) and (NOEC) of various plants that are not registered targets of atrazine in the United States have been evaluated. A great deal has been studied on the non-target effect of atrazine exposure on *Zostera marina*, commonly known as eelgrass. Eelgrass is an aquatic plant native to marine environments in North America (Schwarzschild et al., 1994). Schwarzschild et al., 1994 concluded that atrazine was likely not the factor contributing to the reduction of eelgrass in the Chesapeake Bay due to the sensitivity of whole plants or leaves to atrazine, but not to root-rhizomes systems. Schwarzschild et al. (1994) studied atrazine exposure at environmentally relevant concentrations.

*Vallisneria americana* Michx. (wild celery) was studied in mesocosm systems to delineate chlorophyll flurescence responses to 0 μg/L, 11 μg/L, or 110 μg/L of atrazine following 96 hours exposure (Dantin et al., 2010). A pulse-amplitude modulated (PAM) fluorometer was used in the study to determine changes in the wild celery due to atrazine exposure (Dantin et al., 2010). It is critical to understand the effects of off target chemicals to aquatic plants, since these plants are a necessary factor for an ecosystem to exist in a healthy state. According to Dantin et al. (2010), the electron transport rate values were significantly reduced in the laboratory mesocosms that had exposure to atrazine at 110 μg/L for 96 hours which is likely above the environmentally relevant concentrations of atrazine found in large watersheds.

Research has indicated that atrazine could greatly affect aquatic macrophytes such as *Myriophyllum spicatum* in an off target environmental scenario. Forney et al. (1991) reported a 60% reduction in the growth for *Myriophyllum spicatum* exposed to 1 mg/kg of atrazine in sediments within their mesocosm. The concentration of 1 mg/kg of atrazine in sediments caused a 60% reduction in growth and could be an environmentally relevant concentration for atrazine in the environment (Forney et al., 1991).

The EC50 for leaf growth of *Myriophyllum spicatum* exposed to atrazine was 1,104 μg/L (Solomon et al. 1996 from Davis et al., 1980 and Forney et al., 1981). *Lemna minor* is a freshwater macrophyte that was studied for effects due to atrazine exposure. Following 14d exposure, the NOEC was 10 μg/L and the LOEC was 100 μg/L (Rodgers et al., 1991 cited from within Solomon et al., 1996). Leaf growth of *Elodea canadensis* had a reported EC50 of 80 μg/L after 28 days exposure (Forney et al., 1981). An EC50 for leaf growth was reported at 163 μg/L after 42 days for *Elodea canadensis* (Solomon et al., 1996 from Davis et al. 1980). *Potamogeton pectinatus* displayed oxygen production reductions at 75 μg/L in a study using 21 to 42 day exposures. According to a report by Hoberg et al. (1991), *Lemna gibba* had an EC50 of 180 μg/L after 7 days for frond generation.

| Species | Duration | Comment | Reference |
|---|---|---|---|
| *Zannichellia palustris* | 21–42 d | Oxygen production inhibited at 75 µg/L | [202] |
| *Z. palustris* | 2 h | Photosynthetic efficiency and oxygen production; EC50 = 91 µg/L | [74] |
| *Potamogeton pectinatus* | 21–42 d | Oxygen production inhibited | [202] |
| *P. perfoliatus* | 2 h | Photosynthetic efficiency; EC50 = 77 µg/L | [74] |
| *P. perfoliatus* | 4 h | EC50 = 80 µg/L | [203] |
| *P. perfoliatus* | 7 d | 130 µg/L decreased oxygen production; algacidal concentration = 1,200 µg/L | [21] |
| *P. perfoliatus* | 2–4 wk | 5 µg/L reduced photosynthesis in 2 out of 4 weeks; 50 µg/L reduced photosynthesis in all 4 weeks | [22] |
| *P. perfoliatus* | 4 h | Photosynthesis reduced by 69% at 100 µg/L | [204] |
| *Zostera marina* | 21 d | 10 µg/L reduced growth; LC50 = 100–540 µg/L | [205] |
| *Z. marina* | 21–42 d | Oxygen production inhibited at 650 µg/L | [202] |
| *Z. marina* | 6 h | Net productivity reduced at 100 µg/L; NOEC = 10 µg/L | [206] |
| *Z. marina* | 21 d | LC50 = 100–540 µg/L | |
| *Vallisneria americana* | 9 wk | Growth reduced at 100 µg/L | [70]* |
| *V. americana* | 21–42 d | Oxygen production slightly inhibited at 75 µg/L; significantly reduced at 650 µg/L | [204]* |
| *V. americana* | ? | 4 µg/L reduced tuber development; 8 µg/L reduced growth | [207] |
| *Myriophyllum spicatum* | 28 d | 5 µg/L enhanced photosynthesis; 50 µg/L reduced photosynthesis in 2 out of 4 wk | [22]* |
| *M. spicatum* | 2 h | Photosynthetic efficiency; EC50 = 104 µg/L | [74] |
| *M. spicatum* | 5 d | 50% reduction in number of branches at 3,700 µg/L | [162] |
| *Spartina alterniflora* | 45 d | Minor reductions in growth at 110 µg/L; significant reductions at 1,110 µg/L | [208]* |
| *Ruppia maritima* | 2 h | Photosynthetic efficiency and oxygen production; EC50 = 102 µg/L | [74] |

* References used in the risk characterization.

Table 2. (Solomon et al., 1996)

The table refers effects on saltwater macrophytes exposed to atrazine at vaious time and exposure levels.

Investigations of both freshwater and saltwater macrophytes have revealed varied toxicological reponses for plants that might be exposed to atrazine and that are also vital food sources in an estuarine environment. *Zannichellia palustris* was exposed to 75 µg/L for 21 to 42 days resulting in inhibited oxygen production (Solomon et al.,1996). Photosynthetic efficiency and oxygen production were reduced for *Z. palustris* with an EC50 of 91µg/L (Solomon et al.,1996).

**Zooplankton**

Chemicals can have effects on reproduction of invertebrates. An extension of the research on chemicals affecting reproduction in aquatic invertebrates led to interesting findings with regards to aquatic invertebrates faced with atrazine exposure (Dodson et al., 1999). The water flea, *Daphnia pulicaria* had a sex ratio response to differing concentrations of atrazine at

environmentally relevant levels (Dodson et al., 1999). The high and fast reproduction ability is important for water fleas to survive as a population due to predation (Dodson et al., 1999). The population of water fleas ordinarily reproduces asexually (Dodson et al., 1999). Males are produced usually once a year, initiated by factors such as length of days, changes in food concentrations amongst the population and chemicals produced in a "crowded population" (Dodson et al., 1999). The genetic recombination of the population is achieved when there are males within the population to allow for sexual reproduction. Nevertheless, males are not necessary for the overall proliferation of the population. Inopportune times when males might be induced to become part of the population due to xenobiotic chemical exposure could lead to the demise of the overall population (Dodson 1999). Dodson et al. (1999) discussed past studies revealing a lethal dosage concentration of over 1000 μg/L (Dodson et al., 1999). Other studies have not shown altered sex ratio production in *Daphnia* species. Palma (2009) analyzed effects from .5, 5.0, and 15 mg/L (Palma et al., 2009). They reported that atrazine had relatively little to no effect on male daphnid populations. Atrazine causing little effect in sex ratios within daphnid populations was also found in a publication reported by Olmstead and LeBlanc (2003). The 2003 publication reported no causal effect between atrazine at relatively high concentrations and male populations of daphnids (Olmstead et al., 2003). Olmstead and LeBlanc reported that the choice of algae for the daphnids would likely cause variation in sex ratios. This food choice was different in various laboratories (Olmstead et al., 2003). The atrazine was postulated to be toxic to certain algal food sources to the daphnids, which in turn could be responsible for some of the observed sex ratio changes observed by Dodson et al. (1999).

**Amphibians**

Amphibians have been shown in some laboratory studies and field studies to exhibit altered effects in biological function due to atrazine exposure. The reasoning behind the studies of atrazine linked to sexual mutations in amphibians is due to the established research showing causal links between estradiols and sexual mutations in frogs that have been genetically identified as male (Chang et al., 1956).

Amphibians encompass a wide variety of species, including salamanders, which have been found to be affected by atrazine. Various issues concerning atrazine exposure such as noise and vibrations effecting animals exposed to atrazine have led to speculation that atrazine might cause neurological damage. Larson et al., (1998) found that 250 $\mu$g/L reduced growth following exposure for 86 days in the tiger salamander, *Ambystoma tigrinum*. The embryos of the streamside salamander, *Ambystoma barbouri*, were found to still be viable at 4, 40, and 400 $\mu$g/L, yet noise vibrations effected embryos exposed to 400 μg/L of atrazine (Rohr et al., 2003). The LOEC for the streamside salamander, *A. barbouri*, larvae was 400 μg/L for reduction of size at metamorphosis (Rohr et al. 2004). Rohr et al. ( 2006), found that larval streamside salamander lifespan was shortened significantly due to atrazine exposure in a lab setting at 4 μg/L.

Further toxicity studies have focused on other aspects of health. According to Fernandez and L'Haridon et al. (1993), a 300 μg/L atrazine exposure for 12 days did not alter erythrocytes in the Spanish ribbed newt, *Pleurodeles waltl*. Allran and Karasov et al. (2001) found the NOEC to be 20,000 $\mu$g/L for the hatching of the American toad, *Bufo americanus*.

The immune system of amphibians has been studied in relation to atrazine exposure. Kiesecker et al. (2002) reported that atrazine accentuates the susceptibility of amphibians to acquire parasite infections. This study extended prior research that was not clear on how atrazine might affect amphibians in a real life situation. For example, Fernandez et al. (1993) reported no

observable effect at an atrazine concentration of 300 μg/L for the Spanish Ribbed Newt and alteration of erythrocytes. The NOEC was also apparent in *Pleurodeles waltl* relative to erythrocyte alteration due to atrazine exposure at a concentration above 300 μg/L (L'Haridon et al., 1993).

The effects of atrazine exposure do not usually result in death for amphibians. Despite the lack of mortality as a result of atrazine exposure, other negative effects have been observed in studies evaluating atrazine exposure in amphibians. Nevertheless, there are studies that have delineated lethal dosages of atrazine for various amphibian species far beyond environmentally relevant levels. For example, the LC50 for *Bufo americanus* is above 48,000 μg/L following 8 days exposure according to Birge et al. (1983). The LC50 for an early life stage of *B. americanus* was 26,500 μg/L (Howe et al., 1998).

It is interesting to note that animals exposed to the low concentrations of atrazine sometimes died sooner than did animals exposed to medium or high concentrations in some lab experiments according to Storrs et al. (2004). Although, there has been research indicating that reproductive structures may be harmed due to atrazine exposure, the overall resistance to harmful effects of atrazine in many amphibian species must be acknowledged via a great deal of research. The LOEC due to atrazine exposure resulting in effects on mass and length at metamorphosis for the Gray Tree Frog, *Hyla versicolor,* was reported at 200 μg/L to 2000 μg/L (Diana et al., 2000). Gray Tree Frog mortality due to atrazine exposure was reported at 2000 μg/L following exposure for 28 days (Mazanti et al.; 2003). The American bullfrog, *Rana catesbeiana,* had a LC50 of 410 μg/L after 8 days exposure (Birge et al., 2003). DNA was reported to be damaged at 4,810 μg/L after 24 hours exposure for *Rana catesbeiana* (Clements et al., 1997). The Northern Leopard Frog, *Rana pipiens*, (early life stage) NOEC is 5100 μg/L and LC50 at 47000 μg/L.

An example of a high dosage for an extended period of time involves the Allran et al. (2000) study. Following 138 days of exposure, the Northern Leopard Frog displayed no observable effect concentrations, or NOEC, at 200 μg/L (Allran et al., 2001). They found that the NOEC for egg hatching in the Northern Leopard Frog was 20,000 μg/L following ten days of exposure (Allran et al. 2001).

Storrs et al. (2004) reported that amphibians were found to be affected at contamination levels correlating to EPA maximum contaminant levels for drinking water. Interestingly, Storrs et al. (2004) reported lower concentrations have been seen to have a greater impact on the sexual abnormalities of the physical structure of sexual organs in a number of different amphibians, specifically spring peepers (*Pseudacris crucifer*), American toads (*Bulb americanus*), green frogs (*Rana elamitans*), and wood frogs (*Rana sylvatica*). Early and late developmental stages of effects to amphibians at 3 ppb, 30 ppb and 100 ppb of atrazine exposure have been reported (Storrs et al., 2004).

Atrazine exposure has produced effects on the endocrine system of amphibians, fish, reptiles and human cell lines in the μg/L range within laboratory studies (Hayes et al., 2010). One study indicated that amphibians are effected the most out of any vertebrate species, where potent levels as low as 0.1 ppb reportedly can affect the biological system of the animal (Hayes et al., 2010). It is very concerning that lower concentrations (as low as 0.1 μg/L) can have a more profound impact on the mutation of the developing sexual organs (Hayes et al., 2010).

*Acris crepitans* is a type of amphibian that was studied in the mid 1990's by a team of researchers trying to link atrazine-contaminated water to the phenomenon of intersex frogs found in which both variables are present in the same field location. The Fisher exact test was used to determine if atrazine presence in the environment correlated to the "intersex gonads" of *Acris*

*crepitans* found in the field (Reeder et al., 1998). Water analysis with atrazine presence in conjunction with histological studies of frogs found at those particular sites revealed ($p=0.07$) approaching levels of statistical significance (Reeder et al., 1998). Reeder et al. (1998) reported that it was unclear whether atrazine was to blame for intersexuality in *Acris crepitans*. However, the authors at the time advocated more research for amphibian and atrazine interactions through the "understanding of genetic mechanisms" (Reeder et al., 1998). Reduction at 20 percent due to atresia following dosage at 21 μg/L of atrazine for 48h during sexual differentiation of the ovary (Tavera-Mendoza et al., 2002). The study attempted to uncover the mechanisms as to why sexual differentiation is altered but it seems speculative. Regardless, the germ cells were shown to be affected by atrazine exposure and that in turn shows disruption of reproductive capabilities of amphibians that are exposed to atrazine at critical developmental times of the organism (Tavera-Mendoza et al., 2002).

It has been found that atrazine can affect amphibians at levels as low as 0.1 ppb (Hayes et al., 2010). According to the information available on Web of Science, frogs have the ability to breathe through their skin underwater and this is why they are more susceptible to water contamination (Houck et al., 2006). The emasculation and feminization of amphibians is evident near agricultural areas that use atrazine (Hayes et al., 2010).

The research supporting the effects of atrazine on causing negative effects, such as sexual ambiguity in frogs, has been varied. Oka et al. (2008) found no gonadal changes in *Xenopus laevis* by via laboratory settings. However they did report increased female populations within the sex ratio of froglets at 10 μg/L and 100 μg/L (Oka et al., 2008). Allran et al. (2001) reported hatchability of embryos in laboratory studies on species of *R. pippiens*, *R. sylvatica*, and *B. americanus*.

Research from Tennant et al. (1994) led to the assumption that there was a different biochemical pathway involved that resulted in endocrine disruption in cells. Aromatase increases in biological organisms due to atrazine exposure, which has been the focus of many studies. These studies have focused on determining a causal link between many different types of animals, both invertebrate and vertebrate, for the increased aromatase activity (Hayes et al., 2010).

**Fish**

The lethal dosage of atrazine has been a concern for regulating the pesticide in the United States. However, the past decade has shown a great deal of research to point to other important considerations, such as effects on the reproductive functions of organisms living within environmental settings inundated with atrazine contamination. A study by Tillitt et al. (2010) reported the presence of atrazine in surface and ground waters, notably in the Midwest United States, at concentration ranging from 1μg/L to 25μg/L. This is a concern for fish that may be exposed to atrazine in various waterbodies.

Toxicity of atrazine to freshwater fish has been evaluated and published results have reported that the LC50 is extraordinarily high in most cases. For instance, the LC50 for *Salmo gairdeneri* was found to be 8,800 μg/L following 96 hours exposure (Solomon et al., 1996). It has been reported that *S. gairdneri* has an LC50 of 4,500 μg/L after 96 hour exposure in a different study (Solomon et al.1996). Another toxicity study, cited in Solomon et al 1996, showed an LC50 for *S. gairdneri* of 26,400 μg/L after 72 hours exposure.

*Salvelinus fontinalis* was found to have a LC50 of 6,300 μg/L after 96 hours. *Oncorhynchus kisutch* was found to have a LC50 of 15,000 μg/L after 96 hours exposure (Solomon et al., 1996). *Carassius carassius* was reported to have a LC50 of 76,000 μg/L after

96 hours exposure (Solomon et al., 1996). These studies have been the basis for showing that atrazine is relatively harmless to fish exposed to atrazine since the expected environmental concentrations are much lower than the LC50 for fish. Nevertheless, atrazine was found to amplify gene expression encoding for gonadal aromatase in a particular type of fish (Suzawa et al. 2008). The ratio of male to female offspring of the same species was altered due to prolonged exposure (Suzawa et al., 2008).

The reproduction of certain species of fish has been shown to be effected by atrazine exposure. According to Moore et al. (1998) atrazine exposure had a significant effect on adult salmon reproductive ability (Moore et al., 1998). Tillitt et al., 2010 found that concentrations of 0.5, 5.0, and 50.0 μg/L of atrazine reduced total egg production in fathead minnows (Tillitt 2010). Within the Tillitt (2010) study, the histology of reproductive organs was studied at various times within the 30 day exposure. The tissue of the gonads was examined to evaluate the reproductive stage and the pathological lesions (Tillitt et al., 2010). The water from the fish tank was analyzed by gas chromatography to ensure proper atrazine concentration (Tillitt et al., 2010). Bringolf et al. (2004) investigated vitellogenin as a possible indicator of atrazine acting as an "environmental estrogen". Atrazine exposure inducing aromatase production has been a topic of interest to those concerned with sexual mutations due to off target effects imposed on organisms in the wild. Sanderson et al 2001 reported that fish showed signs of aromatase increases which in turn increased production of endogenous estrogen due to atrazine exposure.

**Reptiles**

In a study on alligators, Crain et al. (1997) reported alteration in aromatase levels due to various concentrations of pesticides (Crain et al., 1997). Varied effects on organisms are often due to the ratios and quantities of the contaminants. Thus, many studies have involved a

combination of pesticides revealing unexpected results for particular species exposed to atrazine and other pesticides in combination experiments (Solomon 1996). Crain et al. (1997) reported induced gonadal adrenal mesonephos in male *Alligator mississippiens* hatchlings exposed to atrazine in water. The atrazine concentrations were 0.14 ppm, 1.4 ppm, and 14.0 ppm. They observed alteration in steroidogenesis in the test subjects (Crain et al., 1997).

Rey et al. (2009) studied crocodile eggs exposed to both atrazine and endosulfan. They reported that atrazine exposure disrupted testicular tissue and altered testosterone levels in the crocodile, *Caiman latirostris* (Rey et al., 2009). The shortage of data focusing on causes of ambiguous genitalia in reptiles should be addressed with more research. Solomon et al. (2008) has stated that there is a paucity of direct data to reflect negative effects of atrazine to reptiles in the wild. Solomon et al. (2008) also cited examples where atrazine did not have effects on reptiles at environmentally relevant levels. For example, Gross et al. (1999) reported that eggs of the red-eared slider turtle, *Pseude myselegans*, had no response in terms of sex ratio at atrazine exposure levels as high as 500 μg/L.

**Conclusion**

In conclusion, atrazine is prevalent in the environment. In addition to the intended effects that atrazine has on photosynthetic processes of target weeds in agriculture, research indicates the possibility of causing negative impacts on other non-target organisms. While atrazine is registered in the United States of America, it is critical that research continue to determine if harm is caused at environmentally relevant concentrations to non target organisms. The impact to non-target organisms includes a wide variety of plants, invertebrates, and vertebrates that are integral parts of many ecosystems and are connected at many different levels. Phytoplankton and aquatic plants have been evaluated to determine the effects of atrazine exposure. In most

instances, both phytoplankton and aquatic plants have both been found to be susceptible to impacts due to the atrazine and its metabolites. Zooplankton have also been found to be susceptible to atrazine. As the complexity of the organism elevates, the studies become more controversial and many questions have arisen for the true validity of the studies in fish, reptiles and amphibians exposed to environmentally relevant concentrations of atrazine.

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